Torque Generation by the Flagellar Rotary Motor

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ABSTRACT A review is given of the structure and dynamics of the flagellar rotary motor. Force-generating elements in a motor driving a tethered bacterium (a cell fixed to the substratum by a single flagellum) exert forces of order 20 pN while moving at speeds of order 1 μm/s. Force-generating elements in a motor driving a flagellar filament in a bundle exert forces some 10-fold lower but move at speeds more than 10-fold higher. The motor torque-speed relationship has been measured over a wide dynamic range. Motors strongly resist being driven backwards and are easily broken.

INTRODUCTION

Some bacteria—I will deal mainly with two closely related enteric species, *Escherichia coli* and *Salmonella typhimurium*—are propelled by rotary engines. These drive long, thin helical filaments that extend out into the external medium and generate the requisite thrust. In *E. coli* and *S. typhimurium*, the power is provided by a protonmotive force (defined as the work per unit charge that a proton can do on crossing the cytoplasmic membrane). In some other organisms that live at high pH or in salt water, it is provided by a sodiummotive force. ATP is not required (Larsen et al., 1974).

A probable structure for this machine, deduced from genetics (cf. Macnab, 1992) and electron microscopy (cf. Francis et al., 1994), is shown in Fig. 1. Structures outside the cell wall include the filament (the propeller), which can be up to about 10 μm long, and the hook (a flexible coupling, or universal joint). Structures embedded in the cell wall comprise the basal body and include several rings and a rod. The outer pair of rings (FlgH, called the L-ring, for lipopolysaccharide, a major component of the outer membrane, and FlgI, called the P-ring, for peptidoglycan, a quasi-rigid polymer that determines the cell's cylindrical shape) is thought to serve as a bushing that gets the rod (FlgB, FlgC, FlgF, and FlgG) through the outer membrane (Berg, 1974). The rod serves as the drive shaft. Gram-positive cells, which do not have an outer membrane, do not have the outer pair of rings. And mutants of *E. coli* in which these rings are missing are motile, provided the hook protein (FlgE) is overproduced (Ohnishi et al., 1987). Therefore, the L- and P-rings are not involved in torque generation. The inner pair of rings, formerly called M (for membranous) and S (for supramembranous), are now called MS, because they are the product of a single gene, *fliF* (Ueno et al., 1992, 1994). An additional ring (called the C-ring, for cytoplasmic) comprises part of a switch complex (FliG, FliM, and FliN) that controls the direction of flagellar rotation. These components are also implicated in torque generation. The interaction of a cytoplasmic protein CheY-P (Che for chemotaxis and P for phosphate) throws the switch into a clockwise (CW) conformation in which the filament, viewed along its helical axis looking toward the cell, spins CW. The null conformation is counterclockwise (CCW). Another cytoplasmic protein, FlgM, turns off the expression of late genes (*fliC, motA, motB, che*, and receptor genes) until it is pumped out of the cell by a competent basal-body-hook complex (Hughes et al., 1993). Therefore, the basal body also includes components (shown by the dashed ring, Fig. 1), involved in protein export, in particular, of axial components of the flagellar structure (cf. Dreyfus et al., 1993).

When wild-type MotA (or MotB) is produced in a *motA* (or *motB*) cell, torque is restored in up to eight equally spaced steps, indicating that there are eight independent force-generating elements, each comprising one or more copies of MotA and MotB (Blair and Berg, 1988). MotA appears to conduct protons from the periplasmic space to the cytoplasm (Blair and Berg, 1990), presumably via a component of the C-ring. MotB is thought to link MotA to the rigid framework of the cell wall (to the peptidoglycan; Chun and Parkinson, 1988; De Mot and Vanderleyden, 1994), but specific connections remain to be identified. Freeze-fracture pictures of wild-type *E. coli* show a circular array of intramembranous particles surrounding a doughnut-like depression; this array is missing in cells deleted for *motA* or *motB* (Khan et al., 1988). So the best working hypothesis is one in which force is generated by the interaction of MotA with the C-ring, coupled to transmembrane proton flow. According to this model, MotA is part of the stator, and the C-ring is part of the rotor. MotA is linked to the rigid framework of the cell wall via MotB, and the C-ring is linked to the flagellar filament via the MS-ring, the rod, and the hook. As noted long ago, the motor must be attached to the wall somewhere, or else the torque that it can generate cannot be applied (Berg, 1974). In short, if you are going to turn the flagellar crank, you must plant your feet.

Much of the effort in my laboratory has gone into determining the dynamic properties of the flagellar motor. This has been done at the level of a single motor by fixing cells

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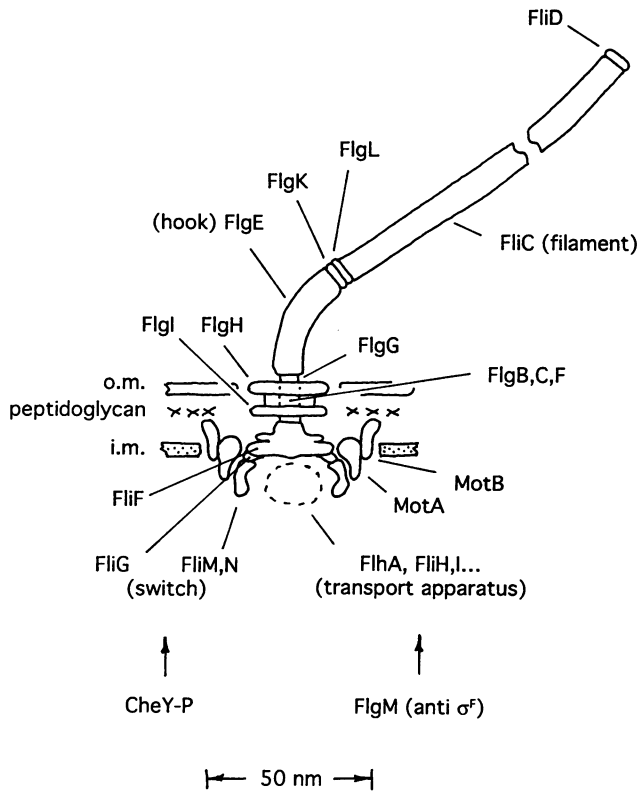

FIGURE 1 The bacterial flagellar motor. Structural components are named after their genes, which fall in the classes *flg, flh, fli* (originally all *fla*, but the alphabet proved too short), and *mot*. Null mutants in the *fla* class are nonflagellate. Null mutants in the *mot* class are nonmotile. o.m., outer membrane; i.m., inner membrane; CheY-P and FlgM, see text.

to the substratum (usually glass) by a single hook or filament, that is, by tethering cells (Silverman and Simon, 1974). We have used a motile *Streptococcus* as well as *E. coli*, because *Streptococcus* can be starved and re-energized, either with a pH gradient or a potassium diffusion potential (Manson et al., 1977, 1980).

A fully energized tethered cell spins relatively slowly, about 10 Hz. The speed is directly proportional to proton-motive force, at least up to a value of −80 mV (Manson et al., 1980; Ravid and Eisenbach, 1984; Conley and Berg, 1984; Khan et al., 1985) and probably beyond (Khan et al., 1990). In *Streptococcus*, there is a threshold for rotation as cells are energized, but not as they are de-energized (Khan et al., 1985). When these cells are energized with a potassium diffusion potential, they show neither a deuterium solvent isotope effect—the torque is the same in D_2O and H_2O—nor a significant thermal effect: the torque changes little over the range 4–38°C (Khan and Berg, 1983). Thus, at low speeds the motor operates close to equilibrium, where rates associated with movement of internal components or proton transfer are not limiting.

Swimming cells spin their flagella more rapidly, at 100 Hz or more. The flagella of a given cell work together in a bundle, and the torque generated by each motor is relatively small (Lowe et al., 1987). With swimming cells, the torque is lower in D_2O (Lowe, 1987; Meister et al., 1987; Blair and Berg, 1990), and higher at higher temperatures (cf. Lowe et al. (1987) and references cited therein). Thus, in this domain, the motor operates far from equilibrium, and rates matter.

Recently, by applying controlled torques to tethered cells using an electrorotation method pioneered by Washizu et al. (1993), we determined the torque-speed relationship for the motor over a wide dynamic range (Fig. 2) (Berg and Turner, 1993). This figure summarizes data on about 90 cells of a smooth swimming (CCW-rotating) strain of *E. coli* studied at three different temperatures, 11.2, 16.2, and 22.6°C. The zero-torque speed, the speed at which the motor shifts from a domain in which it generates thrust to one in which it generates drag, increases markedly with temperature (from about 90 to 140 to 290 Hz, respectively), whereas the slopes of the curves at low torque decline. These curves converge at a common point on the torque axis and are the same except for scaling on the speed axis, which is as expected if temperature changes only rates. Other parameters are temperature-independent.

These data were obtained by comparing the speeds at which a tethered cell spins in the presence of an externally applied torque while the motor is intact to the speeds at which it spins after the motor has been broken (or de-energized by brief treatment with an uncoupler). If one drives the motor backwards (CW), the torque rises rapidly (e.g., to 2 or 3 times the running torque), whereas the motor remains nearly stationary (turns CW at very low speeds, e.g., at 0.01 or 0.1 Hz). Then the motor slips, sometimes obtaining speeds of several Hz, and often breaks catastrophically (suddenly, completely, and irreversibly). Once the motor has been driven rapidly forward beyond the zero-torque speed, breaks are more likely to occur progressively, i.e., in a stepwise manner. In this case, the motor can recover, given time. A catastrophic break probably severs something in series with the hook and the rotor,

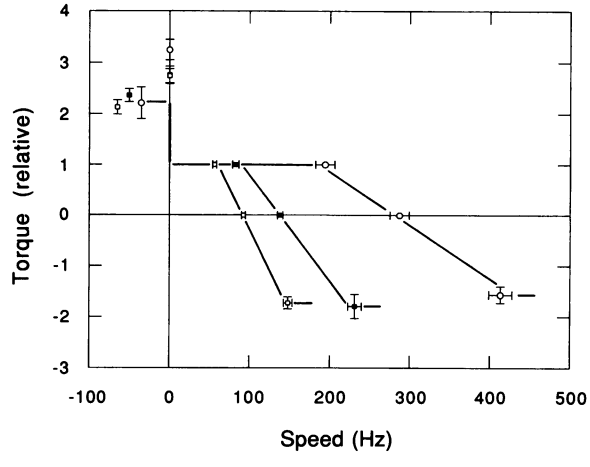

FIGURE 2 Relative torque as a function of speed for a strain of *E. coli* carrying a *cheY* deletion studied at 11.2, 16.2, and 22.6°C (*left* to *right*, respectively). Positive torque and speed are in the forward (CCW) direction. The points near 3 on the torque axis are intercepts of the lines obtained at low torque, high speed (Berg and Turner, 1993, Fig. 6).

e.g., the rod MS-ring connection or the MS-ring C-ring connection, whereas progressive breaks damage parallel elements, e.g., one or more of the MotA-MotB complexes. As seen in the torque restoration experiments, defective (mutant) copies of these proteins can be replaced (Blair and Berg, 1988; Block and Berg, 1984). In any event, the fact that the motor strongly resists backward rotation rules out models with fluid drives. At some point in the working cycle of the motor, there are strong constraints (or strong bonds) that restrict motion of the rotor relative to the stator.

This rigidity speaks for tight coupling, i.e., for the idea that a fixed number of protons carries the motor through each revolution. Experiments with swimming *Streptococcus*, stopped by the addition of anti-filament antibody, suggest a number of order 1200 (Meister et al., 1987). A fundamental prediction of tight coupling is that the stall torque should remain proportional to protonmotive force over the full physiological range. As noted earlier, the data at hand are consistent with this prediction. It remains to be explained how the torque (and thus the efficiency) can be constant over such a wide dynamic range (up to about 60% of the zero-torque speed, Fig. 2).

The absolute torque for a fully energized motor driving a tethered cell (estimated from speed and frictional drag) is about 3×10^{-18} Nm. Assuming that force is applied tangentially to the rotor at a radius of about 20 nm, this implies a total force of about 1.5×10^{-10} N, or about 20 pN for each of eight force-generating elements. For a tethered cell spinning 10 Hz, each generator moves relative to the rotor at a speed of about 1.2 μm/s. If there are 1200/8 = 150 proton-accepting sites around the periphery of the rotor, each generator steps 0.8 nm at a frequency of about 1500 Hz. For the force-generating elements in a motor driving a filament in a bundle, the force is about 10 times smaller and the stepping rate is about 10 times larger.

These numbers are reasonable for tight coupling. In any such machine working close to equilibrium (close to stall), the work done by the force generator in taking the next step (fd, where f is the force exerted and d is the displacement) is equal to the work that a proton can do in moving down its electrochemical gradient ($e\Delta p$, where e is the proton charge and Δp is the protonmotive force). For d = 0.8 nm and Δp = 150 mV, f = 30 pN.

For many years, I have thought of this machine as a thermal ratchet (Berg and Khan, 1983; Khan and Berg, 1983; Meister et al., 1989). MotA engages the rotor near at least two adjacent proton-accepting sites, coupling one to the outside of the cell, via its membrane-spanning domain, and the other to the inside of the cell, via its cytoplasmic domain (cf. Blair and Berg, 1991). Thermal motion of MotA along the periphery of the rotor is allowed or prevented, depending on the protonation of these sites. Barriers to rotation can be quite large; they are not related to the work that a proton can do in moving down its electrochemical gradient. Motion is allowed in either direction if the leading site is protonated and the lagging site is not. Forward motion is strongly favored when the outside of the cell is acid and the inside of the cell is alkaline, or if an electric field drives protons to the bottom of the transmembrane channel (as in Mitchell's proton well; Mitchell, 1969). Under these conditions, backward motion can occur only when one site is protonated at a pH higher than its pK_a while the other site is unprotonated at a pH lower than its pK_a. The probability for this is small, so the motor resists being driven backwards. The motor exerts maximum torque at stall, where the energy available from proton translocation is stored reversibly in springs that link MotA to the cell wall. As the motor turns more rapidly, these springs relax, the torque declines, and proton translocation or diffusion of MotA becomes rate-limiting (see Meister et al., 1989). The time that it takes MotA to diffuse from one set of proton-accepting sites to the next is very small (1 μs or less) because MotA is small (about 3 nm in diameter) and the distance is small (of order 0.8 nm). Speed is not limited by the diffusion of other motor elements. Motor reversal occurs through some global change in the conformation of the C-ring, which brings the force-generating elements into alignment with a different set of proton-accepting sites. For example, one set of sites might be imidazole groups, which are charged when protonated, whereas the second set of sites might be carboxyl groups, which are neutral when protonated. In this event, the constraints on the motion of MotA would depend not on whether the sites are protonated but on whether they are charged.

The successes and failures of all of the models of which I am aware are described briefly elsewhere (Berg and Turner, 1993). One can force the thermal ratchet to generate constant torque over a wide range of speeds by introducing a nonlinear spring, for example, by limiting the extension of a linear spring with a rigid stop. However, this stratagem would render stall torque temperature-sensitive and cause it to saturate at high protonmotive force. Neither seems to be the case. We need to explore new kinetic schemes.

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DISCUSSION

Session Chairperson: Steven Block
Scribe: Michele Moyer

MARCUS SCHAUB (University of Zurich): I believe that a massive flow of protons from outside to inside is necessary to drive the motor so fast. How does the cell ensure a big enough gradient of protons from outside the wall to flow into the cell?

BERG: *E. coli* generates this gradient by respiration or by use of a membrane ATPase, which will take ATP generated at the substrate level and pump protons out though the membrane.

SCHUAB: It must be at a high concentration.

BERG: The protonmotive force is around 150 mV. When *E. coli* are grown at neutral pH the pH gradient is rather small. It's mostly an electrical potential difference. The cytoplasm is heavily buffered, it's about a 50 mM buffer. It takes about 1000 protons per revolution. Depending on how fast you're going you can work out what the proton flux is. It doesn't overwhelm the cell by any means.

SHAHID KHAN (Albert Einstein College of Medicine): I would like you to comment on the relation you observe versus relations published in the literature by other groups, and in particular on the discrepancy between your relation and the relation published by Hotani and co-workers using the electrorotation technique. Also, what is the current status of work in your lab on the torque frequency relation as measured by variable viscous load?

BERG: Those are very technical questions. When the Japanese group did these experiments they did not use very high field strengths. They only drove cells forward up to about 50 Hz, whereas we are drove them forward to about 500 or 1000 Hz. We agree on the constant torque plateau. When they drove cells backwards, they did not see a barrier to rotation. My sense is that they were tethering cells in a very different way, using very long polyhooks. We nailed the filaments down using a sticky filament preparation, then covalently cross-linking them to the substratum. If you don't do that when you work at high field strengths, you blow the cells off the surface. So, the experimental setup is different. There's another report by a group in Nagoya that used an entirely different technique. There's an enormous amount of scatter in that data. If you look at how these curves go, you have several points on the plateau then it comes down. This is all reversible. You can run back and forth several times and get the same answer. What we're doing now to test this proposition is to make minicells. These are cells with a septation